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(54)【発明の名称】　キノン系細胞分化誘導剤

(57)【要約】
【目的】　従来、臨床的有用性の高い医薬品のなかった、細胞分化誘導作用に基づく造血器腫瘍・固形腫瘍などの疾患の治療・改善剤を提供する。
【構成】　従来の癌薬物治療法の基礎となる考え方は、増殖能が異常に高い腫瘍細胞をすべて死滅させるというものであるが、正常細胞に対しても毒性を示すため重篤な副作用が避けられず治療効果にも限界があった。しかし安全性が極めて高いビタミンＫ剤・止血剤等として利用されているメナテトレノンは、意外にも細胞分化誘導作用をも有しており、造血器腫瘍・固形腫瘍等の各種癌・悪性腫瘍に対する、臨床上有用な治療・改善剤となり得る。

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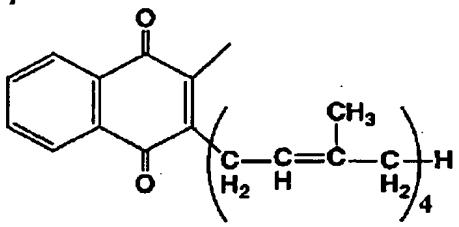
【特許請求の範囲】

【請求項１】　下記化学構造式で表されるメナテトレノンを有効成分とする細胞分化誘導剤。

【化１】



【請求項２】　造血器腫瘍治療剤である請求項１記載の細胞分化誘導剤。

【請求項３】　急性白血病、慢性白血病、悪性リンパ腫、多発性骨髄腫、マクログロブリン血症からなる群より選ばれた疾患の治療・改善剤である請求項２記載の細胞分化誘導剤。

【請求項４】　固形腫瘍治療剤である請求項１記載の細胞分化誘導剤。

【請求項５】　脳腫瘍、頭頸部癌、乳癌、肺癌、食道癌、胃癌、大腸癌、肝癌、胆嚢・胆管癌、膵癌、膵島細胞癌、腎細胞癌、副腎皮質癌、膀胱癌、前立腺癌、睾丸腫瘍、卵巣癌、子宮癌、絨毛癌、甲状腺癌、悪性カルチノイド腫瘍、皮膚癌、悪性黒色腫、骨肉腫、軟部組織肉腫、神経芽細胞腫、ウィルムス腫瘍、胎児性横紋筋肉腫、網膜芽細胞種からなる群より選ばれた疾患の治療・改善剤である請求項４記載の細胞分化誘導剤。

【請求項６】　メナテトレノンを有効成分とする細胞分化誘導作用がその疾患の治療・改善に有効な疾患の治療・改善剤。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、細胞分化誘導（以下、分化誘導）作用に基づく造血器腫瘍・固形腫瘍などの疾患の治療・改善剤に関する。

【０００２】

【発明の背景】わが国における死亡原因の第一位を癌が占めるようになって久しく、しかも患者数は年々増加してきており、有効性および安全性の高い薬剤や治療法の開発が、今や国民・研究者・行政の最大関心事となっている。

【０００３】癌（腫瘍）は発現部位・病理像・症状等により多岐に分類されるが、造血器腫瘍の代表的疾患である白血病は血液細胞（白血球）の腫瘍であり、未分化の各種幼若型白血球細胞の増殖が特徴である。またそれらの中でも、増加している腫瘍細胞が未成熟な芽球であるものを急性白血病、成熟細胞であるものを慢性白血病と分類しており、多岐にわたる臨床症状を呈するが、その多くは、正常造血の抑制に基づく症状と、他臓器への浸潤・圧迫に基づく症状に大別することができる。具体的

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には、正常血球細胞の減少は赤血球減少による貧血・顆粒球減少による感染症や発熱・血小板の減少による出血傾向として現れ、正常造血の抑制は骨髄不全を招く。癌が予後不良な疾患であることは一般によく知られるところであり、これまでにも種々の薬剤や治療方法が検討されてきた。

【０００４】それらの中でも薬物治療法の基礎となる考え方は、腫瘍細胞である白血病細胞をすべて死滅させることにより治療効果を得るというものであり、従ってよりよい治療成績を上げるために、増殖能が異常に高い腫瘍に対し、細胞毒性による殺細胞作用をより強力に示す薬剤の開発や、併用療法、高濃度・多量投与療法などが試みられてきた。しかしこれらの薬剤や治療法は、腫瘍細胞だけに特異的に作用するのではなく、正常細胞に対しても毒性を示すため、心臓・心筋障害、骨髄機能抑制、悪心・嘔吐、神経障害、脱毛等の重篤な副作用が発現し、治療効果にも限界があった。

【０００５】一方、従来の制癌剤と比較して安全性のより高い各種分化誘導剤が、in vitroにおいて腫瘍細胞を成熟細胞へ分化誘導する事実は知られており、分化誘導療法への期待が集まっていたが、残念ながら従来の分化誘導剤では臨床での有用性が認められていなかった。しかし1988年にヒュン(Huang) らが、オールトランス－レチノイン酸（以下、ATRA）が急性前骨髄性白血病（以下、APL)患者に対し100%に近い完全寛解をもたらした臨床成績を報告して以来［ブラッド(Blood), 72;567-572, 1988.]、世界各国においてその効果が再確認され、造血器腫瘍のみならず固形腫瘍を含めた広い範囲の癌に対する分化誘導療法に期待が高まりつつある。

【０００６】

【従来技術】前述のように、ATRAが臨床において APLに有効であることは、ヒュン(Huang)ら［ブラッド(Blood), 72,567-572,1988.]を始め、キャステン(Castaigne) ら［ブラッド(Blood), 76,1704-1709,1990.]、ワーレル(Warrell) ら［ニューイングランド・ジャーナル・オブ・メディスン(New Engl.J.Med.),324,1385-1393,1991.]など、多く研究者が報告している。

【０００７】またオルソン(Olsson)らは、ビタミンD_3の生理活性型代謝物である 1α,25-ジヒドロキシコレカルシフェロール（以下、活性$V.D_3$）が、ヒト・リンパ腫培養細胞系（U937）において分化誘導作用を有することを報告している［キャンサー・リサーチ(Cancer Res.), 43(12Pt1),5862-5867,1983.]。これより分化誘導作用を有する活性$V.D_3$誘導体の開発も盛んに行われるようになり、例えば特開昭61-33165号公報には24－アルキルーデヒドロビタミンD_3 誘導体が抗腫瘍作用を有することが、また特開昭 61-140560号公報には20－オキサ-21-ノルービタミンD_3誘導体が分化誘導作用を有することが、それぞれ開示されている。

【０００８】ツァン(Zhang) らは、ブファリン(Bufali

n）がヒト白血病細胞の培養細胞系であるHL60、U937および ML1において分化誘導作用を示したことを報告している［バイオケミカル・アンド・バイオフィジカル・リサーチ・コミュニケーションズ(Biochem.Biophys.Res.Commun.),178(2),686-693,1991.およびキャンサー・リサーチ(Cancer Res.),52(17),4634-4641,1992.］。

【０００９】また上記以外にも分化誘導作用を有する化合物として、バカラーニ(Baccarani) らはシトシン・アラビノシド(Ara-C) を［ブリティッシュ・ジャーナル・オブ・ヘマトロジー(Br.J.Haematol.),42,485-487,1979.]、モーリン(Morin) らはアクラシノマイシンＡを［キャンサー・リサーチ(Cancer Res.),44,2807-2812,1984.］、森屋らはインターフェロンーαを［臨床血液,32,170-172,1991.]に報告している。

【００１０】石倉らは、マウス骨髄球性白血病の培養細胞系を用いて、ゲラニル・ファルネソール（3,7,11,15,19ーペンタメチル-2､6､10､14､18-エイコサペンタエンー1-オール）が分化誘導作用を有することを報告している［ロイケミア・リサーチ(Leukemia Res.),8(5),843-852,1984.］。

【００１１】

【本発明が解決しようとする問題点】ATRAおよびその誘導体は、皮膚癌や難治性皮膚角化疾患である乾癬の治療に利用されているが、脂溶性が極めて高いため、長期間投与すると肝臓の肥大・神経異常・食欲不振・嘔吐・脱毛・そう痒感等のビタミンＡ過剰症状を発現しやすいことが広く知られており、かつ投与を中止しても肝臓や組織に長期間残留するため、副作用が一度発現すると長期間消失しない重大な欠点がある。またATRAが APLに有効であることは前述の通りであるが、 APLが全白血病患者中に占める割合は約5%と非常に少なく、他の多くのタイプの急性白血病患者にはほとんど無効であった。さらに寛解後も投与を中止すると再発しやすい問題もあった。

【００１２】ビタミンD_3 誘導体は骨粗鬆症などの治療に利用されているが、腸管でのカルシウム吸収および腎臓におけるカルシウム再吸収を促進するので、投与量が過剰になると高カルシウム血症を引き起こし、石灰沈着に起因する腎臓障害や消化器障害をもたらすことが知られている。このため投与期間中は定期的に血清カルシウム値を検査しなければならず、臨床では非常に使いにくい問題点がある。さらにビタミンD_3 誘導体の分化誘導作用は、ヒト前骨髄球性白血病の培養細胞系であるHL60には有効であるが、他のタイプのモデルにおいては有効性が認められていない。

【００１３】ブファリンは臨床には応用されていないため、その安全性に関して全く不明であり、ヒトでの有用性を予測することはできなかった。

【００１４】さらにシトシン・アラビノシドやアクラシノマイシンＡも安全性上の問題から国内では薬剤として許可されておらず、インターフェロンーαの抗腫瘍作用も期待されたほどではなかった。

【００１５】ゲラニル・ファルネソールの分化誘導作用に関する評価結果はマウス白血病細胞培養細胞系におけるものである。その後ヒト白血病細胞培養細胞系での評価結果は全く報告されていないので、種の異なる細胞間での薬剤感受性の差を考慮すると、ヒトでの有効性は一切不明であった。

【００１６】このように、各種癌に対して優れた有効性と安全性を兼ね備えた薬剤はないのが現状であり、臨床で広範囲の癌に対し有用性の高い医薬品の開発が強く望まれていた。

【００１７】

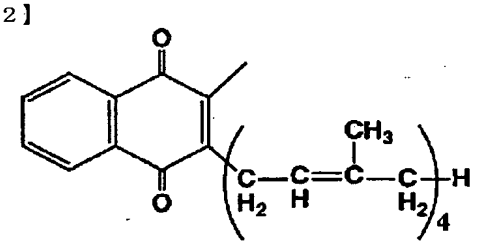
【課題を解決するための手段】本発明にかかる、メナテトレノン（ビタミンK_2）は止血ビタミンとして、ビタミンK 欠乏による下記疾患および症状の改善作用を有する化合物として公知であり、医薬品として臨床で広く使用されている。

(1) 胆道閉塞・胆汁分泌不全による低プロトロンビン血症
(2) 新生児低プロトロンビン血症
(3) 分娩時出血
(4) クマリン系抗凝血薬投与中に起こる低プロトロンビン血症

【００１８】さらにメナテトレノン自身の副作用は少なく、極めて安全性の高い化合物でもある。本発明者らは、メナテトレノンが、優れた生理活性とヒトや動物への安全性が高いという要件を兼ね備えていることに着目し、永年他の疾患への有効性も検討してきた。その結果、意外にもメナテトレノンが分化誘導作用も有しており、造血器腫瘍・固形腫瘍などの各種癌に対する治療・改善剤として所期の目的を達成できることを見い出し本発明を完成した。メナテトレノンは下記化学構造式で表される。

【００１９】

【化２】



【００２０】メナテトレノンは分子内に４組の二重結合を有し、そのうち３組については２種類づつ（Ｅ体、Ｚ体）、計８種類の幾何異性体が存在し、本発明においてはいずれの異性体でもよく限定されないが、オールトランス体がより好ましい。また本発明ではこれらの幾何異性体のうち１種類を単独で用いてもよいし、２種類以上の混合物を用いてもよく限定されない。さらにメナテトレノンには、天然抽出物と合成品とがあるが、由来も限

定されない。なおメナテトレノンは、医薬品、化粧品、食品、工業原料などとして広く販売されており、容易に入手することができる。

【００２１】従って本発明の目的は、分化誘導作用を有する臨床的有用性の高い、各種癌に対する治療・改善剤を提供することにある。具体的にはメナテトレノンを有効成分とする、造血器腫瘍・固形腫瘍等の各種癌・悪性腫瘍の治療・改善剤、および本化合物の分化誘導作用が有効な疾患の治療・改善剤に関する。ここで造血器腫瘍の具体的疾患名の一例としては、例えば急性白血病、慢性白血病、悪性リンパ腫、多発性骨髄腫、マクログロブリン血症などを挙げることができ、また固形腫瘍としては、例えば脳腫瘍、頭頸部癌、乳癌、肺癌、食道癌、胃癌、大腸癌、肝癌、胆嚢・胆管癌、膵癌、膵島細胞癌、腎細胞癌、副腎皮質癌、膀胱癌、前立腺癌、睾丸腫瘍、卵巣癌、子宮癌、絨毛癌、甲状腺癌、悪性カルチノイド腫瘍、皮膚癌、悪性黒色腫、骨肉腫、軟部組織肉腫、神経芽細胞腫、ウィルムス腫瘍、胎児性横紋筋肉腫、網膜芽細胞種などを挙げることができるが、本発明の対象疾患がこれらに限定されないことは言うまでもない。

【００２２】また本発明においては上記治療・改善剤としての有効性に加え、長期間投与しても極めて高い安全性が期待できることから、長期間治療を続けることが可能となり、癌患者のクオリティー・オブ・ライフの改善に大きく貢献する発明であると言える。

【００２３】次にメナテトレノンの安全性を示すために、急性毒性試験試験結果（LD_{50} 値）を示す。

【表１】

メナテトレノンの急性毒性（mg/Kg）

動物種	性別	経口	皮下	腹腔内
マウス (ICR系)	♂	＞5,000	＞5,000	＞5,000
	♀	＞5,000	＞5,000	＞5,000
ラット (SD系)	♂	＞5,000	＞5,000	＞5,000
	♀	＞5,000	＞5,000	＞5,000

【００２４】表１から、メナテトレノンの極めて高い安全性が明らかである。さらに前述のようにメナテトレノンはすでに医薬品として広く臨床で使用されており、その安全性は確認されている。

【００２５】投与剤型としては、例えば散剤、細粒剤、顆粒剤、錠剤、被覆錠剤、カプセル剤などの経口製剤、注射製剤および外用剤（経皮製剤）が挙げられる。製剤化の際には、通常の製剤担体を用いて常法により製造することができる。

【００２６】すなわち経口製剤を製造するには、メナテトレノンと賦形剤、さらに必要に応じて結合剤、崩壊剤、滑沢剤、着色剤、矯味矯臭剤などを加えた後、常法により散剤、細粒剤、顆粒剤、錠剤、被覆錠剤、カプセル剤等とする。

【００２７】賦形剤としては、例えば乳糖、コーンスターチ、白糖、ブドウ糖、マンニトール、ソルビトール、

結晶セルロース、二酸化ケイ素などが、結合剤としては、例えばポリビニルアルコール、ポリビニルエーテル、メチルセルロース、エチルセルロース、アラビアゴム、トラガント、ゼラチン、シェラック、ヒドロキシプロピルメチルセルロース、ヒドロキシプロピルセルロース、ポリビニルピロリドン、ポリプロピレングリコール・ポリオキシエチレン・ブロックポリマー、メグルミンなどが、崩壊剤としては、例えば澱粉、寒天、ゼラチン末、結晶セルロース、炭酸カルシウム、炭酸水素ナトリウム、クエン酸カルシウム、デキストリン、ペクチン、カルボキシメチルセルロース・カルシウム等が、滑沢剤としては、例えばステアリン酸マグネシウム、タルク、ポリエチレングリコール、シリカ、硬化植物油等が、着色剤としては医薬品に添加することが許可されているものが、矯味矯臭剤としては、ココア末、ハッカ脳、芳香散、ハッカ油、竜脳、桂皮末等が用いられる。これらの錠剤・顆粒剤には糖衣、その他必要により適宜コーティングすることはもちろん差支えない。

【００２８】また注射用製剤を製造する際には、メナテトレノンにpH調整剤、溶解剤、等張化剤などと、必要に応じて溶解補助剤、安定化剤などを加えて、常法により製剤化する。

【００２９】外用剤を製造する際の方法は限定されず、常法により製造することができる。すなわち製剤化にあたり使用する基剤原料としては、医薬品、医薬部外品、化粧品等に通常使用される各種原料を用いることが可能である。

【００３０】使用する基剤原料として具体的には、例えば動植物油、鉱物油、エステル油、ワックス類、高級アルコール類、脂肪酸類、シリコン油、界面活性剤、リン脂質類、アルコール類、多価アルコール類、水溶性高分子類、粘土鉱物類、精製水などの原料が挙げられ、さらに必要に応じ、pH調整剤、抗酸化剤、キレート剤、防腐防黴剤、着色料、香料などを添加することができるが、本発明にかかる外用剤の基剤原料はこれらに限定されない。また必要に応じて他の分化誘導作用を有する成分、血流促進剤、殺菌剤、消炎剤、細胞賦活剤、ビタミン類、アミノ酸、保湿剤、角質溶解剤等の成分を配合することもできる。なお上記基剤原料の添加量は、通常外用剤の製造にあたり設定される濃度になる量である。

【００３１】本発明におけるメナテトレノンの臨床投与量は、症状、重症度、年齢、合併症などによって異なり限定されず、また化合物の種類・投与経路などによっても異なるが、通常成人１日あたり10mg～10g であり、好ましくは50mg～5gであり、さらに好ましくは 100mg～1gであり、これを経口、静脈内または経皮投与する。

【００３２】次に本発明を具体的に説明するため以下に実施例を掲げるが、本発明がこれらに限定されないことは言うまでもない。

【００３３】

7　　8

【実施例】

実施例１　顆粒剤

【００３４】

【表２】

<処方>

	原料	配合量（ｍｇ）
1)	メナテトレノン	１００．０
2)	無水ケイ酸	１００．０
3)	D-マンニトール	４５０．０
4)	ヒドロキシプロピルセルロース	４０．０
5)	dl－α－トコフェロール	０．２
6)	タルク	１０．０
7)	乳糖	約　３００．０

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【００３５】実施例２　錠剤

【００３６】

【表３】

<処方>

	原料	配合量（ｍｇ）
1)	メナテトレノン	１０．０
2)	ヒドロキシプロピルセルロース	５０．０
3)	乳糖	１００．０
4)	トウモロコシデンプン	２０．０
5)	無水ケイ酸	３．０
6)	ステアリン酸マグネシウム	０．２
7)	マクロゴール６０００	３．０
8)	ポリビニルピロリドン	０．６
9)	アラビアゴム末	３．０
10)	沈降炭酸カルシウム	４．０
11)	酸化チタン	１０．０
12)	タルク	１５．０
13)	白糖	約　６０．０

【００３７】実施例３　注射剤

【００３８】

【表４】

<処方>

	原料	配合量（重量%）
1)	メナテトレノン	１．０
2)	ポリオキシエチレンソルビタンモノオレート	３．５
3)	D-ソルビトール	５．０
4)	リン酸二水素ナトリウム（NaH_2PO_4）	０．０８
5)	リン酸水素ナトリウム　（Na_2HPO_4）	０．０７
6)	精製水	加えて１００．０

【００３９】実施例４　外用剤

【００４０】

【表５】

<処方>

	原料	配合量（重量%）
1)	メナテトレノン	１．０
2)	スクワラン	１０．０
3)	ミリスチン酸イソプロピル	７．０
4)	ベヘニルアルコール	１．０
5)	セトステアリルアルコール	５．５
6)	ステアリン酸モノグリセリン	２．０
7)	d－α－トコフェロール	０．０５
8)	ＰＯＥ（２０）モノステアリン酸ソルビタン	２．０
9)	キサンタンガム	０．１
10)	１、３－ブチレングリコール	２．０
11)	グリセリン	３．０
12)	D-ソルビトール	５．０
13)	パラベン	０．２
14)	精製水	加えて１００．０

【００４１】

【発明の効果】次に本発明化合物の分化誘導剤としての有用性を示すため、マウスB16 メラノーマ細胞および各種ヒト白血病培養細胞系に対する効果実験例を挙げる。

なお実験に用いたヒト白血病培養細胞系は以下の通りである。

(1) HL60；ヒト前骨髄性白血病細胞
(2) U937；ヒト単芽球様白血病細胞
(3) ML1 ；ヒト骨髄芽球様白血病細胞
(4) K562；ヒト骨髄赤芽球白血病細胞

【００４２】実験１　マウスB16 メラノーマ細胞に対するメナテトレノンの分化誘導作用

（方法）マウス由来 B16メラノーマ細胞に対するメナテトレノンの分化誘導作用を、メラニン生成能を指標として評価した。すなわちB16ﾒﾗﾉｰﾏ細胞を継代培養後、 2×10^4 セル/ml になるよう 10%FCS MEM*に加え培養用シャーレ（φ＝10cm）にて24時間培養した。培養後、各試料が毒性を示さなかった濃度（ 1.0×10^{-6} M）に調製した 10%FCS MEM で培地交換を行った後、同条件で 5日間培養した。培養後、等張緩衝塩類溶液［日水製薬製、商品名；Dulbecco's PBS(-) ］で洗浄し、0.25% トリプシン／エチレンジアミンテトラ酢酸(EDTA)溶液を用いて細胞を集め、さらに上記等張緩衝塩類溶液で再び洗浄した後、遠心分離(100G)して細胞を得た。（ 10%FCS MEM*；標準培地に 10%ウシ胎仔血清、ペニシリン、ストレプトマイシンおよび炭酸水素ナトリウムを添加した培地）

【００４３】得られた細胞に1mM-フェニルメチルスルホニルフルオリド(PMSF) 1mlを添加したリン酸緩衝液を加えた後、及川らの方法（エール・ジャーナル・オブ・バイオロジカル・メディスン[Yale J.Biol.Med.], 46,500-507,1973.）にしたがって総メラニン量を吸光度（λ＝400nm ）で測定し評価した。

【００４４】表６に、マウス由来 B16メラノーマ細胞に対するメナテトレノンの分化誘導作用を示す。

【００４５】

【表６】

マウスＢ１６メラノーマ細胞に対するメナテトレノンの分化誘導作用

試　料	培養細胞タンパク量あたりの総メラニン量（%）
メナテトレノン	８５
コントロール	１００

【００４６】表６から明らかなように、 1.0×10^{-6} M のメナテトレノンにて５日間培養処理した B16メラノーマ細胞の蛋白量あたりの総メラニン量（ユーメラニンおよびフェオメラニン）は、コントロール培養細胞に比べ約 15%低下した。この時の細胞内チロシナーゼ量は、メナテトレノン処理により明らかに減少したことが SDS電気泳動法により確認された。

【００４７】上記の結果はメナテトレノンの固形腫瘍に対する有効性を示すものであり、メナテトレノンの造血器腫瘍の分化誘導のみに止まらない幅広い適応性を示唆するものである。

【００４８】実験２～５　各種ヒト白血病培養細胞系に対するメナテトレノンの分化誘導作用

（方法）実験２～５にかかる分化誘導作用の評価は、文献に記載されている方法［中谷ら、キャンサー・リサーチ(Cancer Res.),48,4201-4205,1988.］に従って行い、下記分化誘導マーカーについて測定・評価した。

(1) 正常細胞への分化誘導マーカーであるニトロブルーテトラゾリウム（以下、 NBT）還元能は、細胞を NBT試薬と37℃で40分間インキュベートし、還元されて生じたフォルマザンを顕微鏡で観察して評価した。

(2) 死細胞の割合（細胞の viability）は、トリパンブルー試薬で染色された細胞を死細胞とし、全体の細胞数に対する百分率を算出した。

【００４９】（結果）

実験２　ヒト前骨髄性白血病細胞 HL60 に対する分化誘導作用

次にヒト骨髄芽球様白血病細胞 HL60 に対する、メナテトレノンの濃度と分化誘導作用の関係を図１に示す。

【００５０】

【図１】

【００５１】図１から明らかなように、メナテトレノン濃度の増加と共に NBT還元能（分化誘導能）は増加し、 1.0×10^{-5} M のメナテトレノン処理では約 57%の細胞に分化が認められた。一方細胞数はメナテトレノン濃度の増加と共に減少し、同じく 1.0×10^{-5} M のメナテトレノン処理でコントロールの約 48%に減少し、増殖阻害作用も認められた。死細胞数の増加は、 1.0×10^{-5} M までコントロールと比較してわずかであり、細胞毒性はほとんど認められなかった。従ってメナテトレノンは、細胞毒性に基づかずに特徴的にHL60細胞の分化を誘導することが明らかである。

【００５２】実験３　ヒト単芽球様白血病細胞 U937 に対する分化誘導作用

ヒト単芽球様白血病細胞 U937 に対する、メナテトレノン濃度と分化誘導作用の関係を図２に示す。

【００５３】

【図２】

【００５４】図２から明らかなように、メナテトレノン濃度の増加と共に NBT還元能（分化誘導能）は増加し、 1.0×10^{-6} M のメナテトレノン処理では約 84%の細胞に分化が認められた。一方細胞数はメナテトレノン濃度の増加と共に減少し、同じく 1.0×10^{-6} M のメナテトレノン処理でコントロールの約 61%となり、増殖阻害作用も認められた。死細胞数は、 5.0×10^{-6} M までコントロールと比較して差は認められず、細胞毒性は認められなかった。従ってメナテトレノンは、細胞毒性に基づかずに特徴的にU937細胞の分化を誘導することが明らかであ

る。

【００５５】実験４　ヒト骨髄芽球様白血病細胞 ML1に対する分化誘導作用

ヒト骨髄芽球様白血病細胞 ML1に対する、メナテトレノン濃度と分化誘導作用の関係を図３に示す。

【００５６】

【図３】

【００５７】図３から明らかなように、メナテトレノン濃度の増加と共に NBT還元能（分化誘導能）は増加し、1.0×10^{-6} M のメナテトレノン処理で約 84%の細胞に分化が認められた。一方細胞数はメナテトレノン濃度の増加と共に減少し、同じく 1.0×10^{-6} M のメナテトレノン処理でコントロールの約 61%となり、増殖阻害作用も認められた。死細胞数は、1.0×10^{-6} M までコントロールと比較して差は認められず、細胞毒性は認められなかった。従ってメナテトレノンは、細胞毒性に基づかずに特徴的に ML1細胞の分化を誘導することが明らかである。

【００５８】実験５　ヒト骨髄赤芽球白血病細胞 K562に対する分化誘導作用

ヒト骨髄赤芽球白血病細胞 K562 に対する、メナテトレノン濃度と分化誘導作用の関係を図４に示す。

【００５９】

【図４】

【００６０】図４から明らかなように、メナテトレノン濃度の増加と共に NBT還元能（分化誘導能）は明らかに増加し、1.0×10^{-6} M のメナテトレノン処理では 60%の細胞に分化が認められた。一方細胞数は 1.0×10^{-5} M 以上の高濃度において減少し、細胞増殖抑制作用は弱かった。死細胞数は、2.0×10^{-5} M までコントロールと比較して差は認められず、細胞毒性は認められなかった。この結果より、メナテトレノンは前記のようにHL60、U937、 ML1の各細胞に対するほど強くはないが、ヒト骨髄赤芽球白血病細胞K562細胞の分化も誘導することが明らかである。

【００６１】上記実験例の結果から、メナテトレノンは 10^{-6} ～10^{-5} M 濃度において、発生段階の異なる各種ヒト白血病細胞の分化を誘導することが明らかである。しかも取り分け骨髄芽球様白血病細胞(HL60)、単芽球様白血病細胞(U937)、骨髄芽球様白血病細胞(ML1) に対する効果がより顕著であることが特徴的であり、骨髄赤芽球白血病細胞に対する分化誘導能も有している。

【００６２】実験６　各種ヒト白血病培養細胞系における、メナテトレノンが他の分化誘導マーカーに与える効果

さらに NBT還元能と細胞数以外には、以下のマーカーが分化誘導の指標として利用されており、それぞれ次のような意味を有する。

(1) AS-D－クロロアセテートエステラーゼ活性；顆粒球への分化

(2) α－ナフチルアセテートエステラーゼ活性；単球（マクロファージ）への分化

(3) 貪食能；正常白血球細胞への分化

(4) F_c レセプター数；顆粒球・単球への分化

【００６３】（方法）上記マーカーの測定は、(1)(2)は前記文献［中谷ら、キャンサー・リサーチ(Cancer Res.),48,4201-4205,1988.］記載の方法に従った。(3)は、細胞とポリスチレンラテックスビーズを37℃で４時間インキュベートし、10個以上のビーズを取り込んだ細胞数をカウントした。細胞の viability（生細胞の割合）はトリパンブルー試薬で染色されない細胞を生細胞とし、全体の細胞数に対する百分率を貪食能として算出した。(4) はモレキュラー・イムノロジー(Molecular Immunology),25(11),1159-67,1988. に記載された方法に従った。

【００６４】（結果）次にメナテトレノンが、上記の分化誘導マーカーに与える効果を表７に示す。

【００６５】

【表７】

その他の分化誘導マーカーに対するメナテトレノンの効果

細胞	ﾒﾅﾃﾄﾚﾉﾝ 濃度 (M)	ASD-ｸﾛﾛｱｾﾃｰﾄ ｴｽﾃﾗｰｾﾞ活性	α－ﾅﾌﾁﾙｱｾﾁﾙ ｴｽﾃﾗｰｾﾞ活性	貪食能	Fc ﾚｾﾌﾟﾀｰ 数
HL60	ｺﾝﾄﾛｰﾙ	36.5± 5.6	12.6± 1.8	12.8± 3.1	1.6± 0.2
	1×10^{-6}	37.5± 2.0	15.3± 2.2	21.4± 4.4	1.8± 0.1
	2×10^{-6}	27.6± 3.4	22.7± 3.6	37.5±13.5	1.7± 0.1
U937	ｺﾝﾄﾛｰﾙ	7.2± 2.2	6.2± 1.7	12.5± 2.9	1.2± 0.3
	1×10^{-6}	13.2± 0.2	13.9± 4.2	17.8± 1.2	12.5±10.7
	2×10^{-6}	10.0± 4.2	33.1±10.3	18.2± 6.7	8.8± 2.7
ML1	ｺﾝﾄﾛｰﾙ	11.3± 4.2	9.7± 2.3	8.9± 3.5	2.5± 0.9
	2×10^{-6}	27.8± 2.6	14.3± 3.2	34.4±12.9	31.4± 5.0
	1×10^{-5}	38.6± 7.4	34.8±14.4	40.0± 0.0	13.0± 0.0
K562	ｺﾝﾄﾛｰﾙ	6.2± 0.4	5.3± 2.5	15.2± 7.9	2.8± 2.0
	1×10^{-6}	6.7± 3.8	2.6± 1.3	19.5± 3.0	3.6± 1.9
	2×10^{-6}	6.9± 3.2	4.0± 0.9	10.2± 3.0	6.7± 3.2

【００６６】表７から明らかなように、メナテトレノン処理により、ヒト骨髄芽球様白血病細胞 HL60 およびヒト単芽球様白血病細胞 U937 において、α－ナフチルアセテートエステラーゼ活性および貪食能が正常化しており、これらのヒト白血病細胞が単球（マクロファージ）へ分化誘導されたことがわかる。またヒト骨髄芽球様白血病細胞 ML1においては、すべての分化誘導マーカーが改善されており、顆粒球および単球へ分化誘導されたことがわかる。これらの結果より、メナテトレノンはタイプの異なる各種ヒト白血病培養細胞に対し、広く分化誘導能を有していることが明らかである。

【００６７】

【図面の簡単な説明】

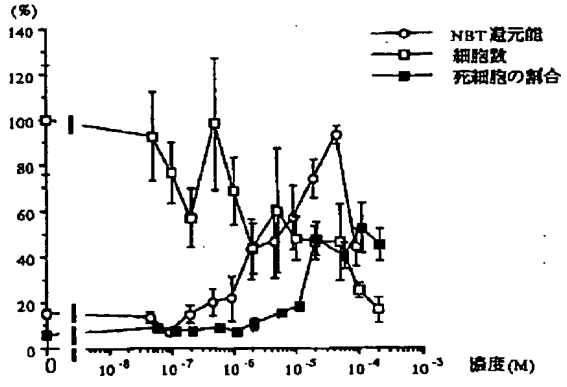
【図１】　前骨髄性白血病細胞 HL60 に対するメナテトレノン濃度と分化誘導作用との関係を示した図である。（各群とも n= ３、平均±標準誤差で示す）

【図２】　ヒト単芽球様 U937 細胞に対するメナテトレノン濃度と分化誘導作用との関係を示した図である。（各群とも n= ３、平均±標準誤差で示す）

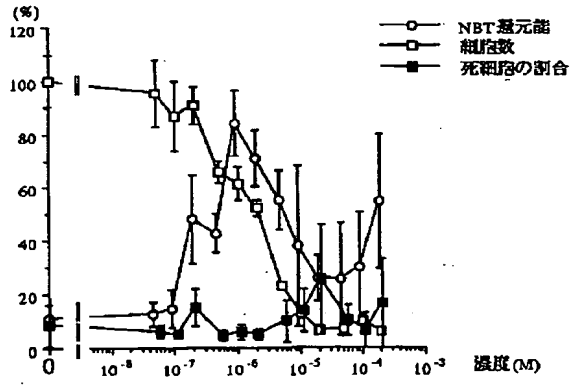
【図３】　ヒト骨髄芽球様白血病細胞 ML1に対する、メナテトレノン濃度と分化誘導作用の関係を示した図である。（各群とも n= ３、平均±標準誤差で示す）

【図４】　ヒト骨髄赤芽球白血病細胞 K562 に対するメナテトレノン濃度と分化誘導作用との関係を示した図である。（各群とも n= ３、平均±標準誤差で示す）

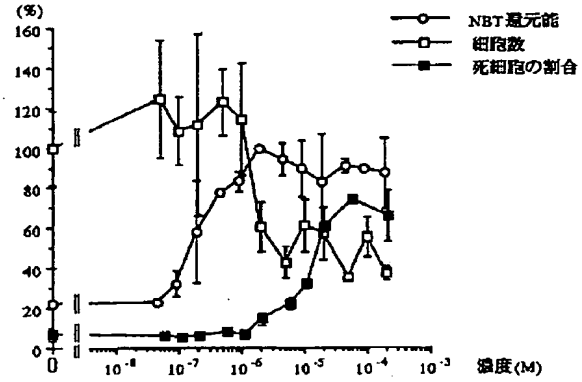
【図１】



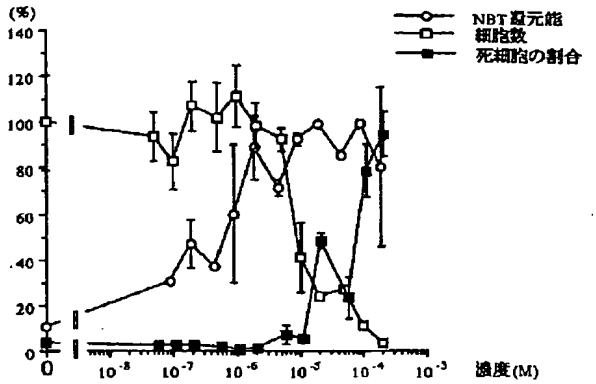
【図２】



【図３】



【図４】



プロントページの続き

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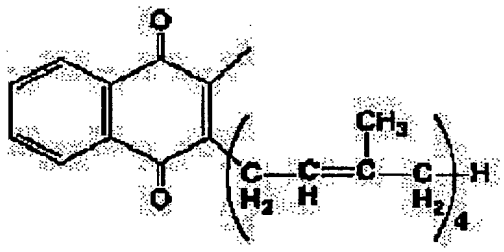
(54) QUINONE-BASED CELL DIFFERENTIATION INDUCER

(57)Abstract:
PURPOSE: To obtain a medicine, containing menatetrenone as an active ingredient, having cell differentiation inducing action and useful for treating tumors of hematopoietic organs, solid tumors, etc.
CONSTITUTION: The quinone-based cell differentiation inducer contains menatetrenone (vitamin K2) expressed by the formula as an active ingredient. The menatetrenone is well-known as a hemostatic vitamin and clinically widely used as a medicine with hardly any side effects, has high safety and further cell differentiation inducing action and can be formulated into a dosage form such as an oral agent, a parenteral injection or an external percutaneous agent. The daily dose is 10mg to 1g, preferably 100 mg to 1g for an adult. The inducer is useful for tumors of hematopoietic organs such as acute leukemia, chromic leukemia, malignant lymphoma, multiple myeloma or macroglobulinemia and solid tumors such as cerebral tumor, head and neck cancer, mammary cancer, lung cancer, esophageal carcinoma, gastric cancer, carcinoma of the colon, cancer of the liver, gallbladder and bile duct cancer, cancer of the pancreas, islet cell cancer, renal cell carcinoma, adrenocortical cancer, bladder cancer, prostatic cancer, testicular tumor, ovarian cancer, uterine cancer, villous cancer, thyroid cancer, malignant carcinoid tumor, carcinoma cutaneum, malignant melanoma or osteosarcoma.



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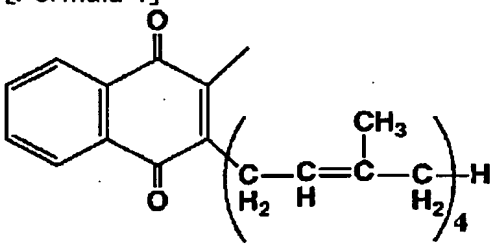
[Date of extinction of right]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The cell differentiation inducer which makes an active principle the menatetrenone expressed with the following chemical structure type.
[Formula 1]



[Claim 2] The cell differentiation inducer according to claim 1 which is a hematopoietic organ oncotherapy agent.

[Claim 3] Acute leukemia, chronic leukemia, a malignant lymphoma, a multiple myeloma, the cell differentiation inducer according to claim 2 that is therapy / improvement agent of the disease chosen from the group which consists of a macroglobulinemia.
[Claim 4] The cell differentiation inducer according to claim 1 which is a solid oncotherapy agent.
[Claim 5] A brain tumor, a head and neck cancer, a breast cancer, lung cancer, an esophagus cancer, gastric cancer, colon cancer, hepatic carcinoma, a gallbladder and a cholangioma, a pancreatic cancer, islet cell cancer, renal cell carcinoma, adrenal cortical adenocarcinoma, vesical cancer, a prostatic cancer, the orchioncus, an ovarian cancer, a uterine cancer, a choriocarcinoma, a thyroid cancer, a carcinoid-type-bronchial-adenoma neoplasm, skin carcinoma, a malignant melanoma, an osteosarcoma, a soft tissue sarcoma a neuroblastoma, a Wilms' tumor, the embryonal rhabdomyosarcomas, the cell differentiation inducer according to claim 4 that is therapy / improvement agent of the disease chosen from the group which consists of a retinoblast kind
[Claim 6] Therapy / improvement agent of a disease with the cell differentiation induction operation effective in a therapy and an improvement of the disease which makes menatetrenone an active principle.

[Translation done.]

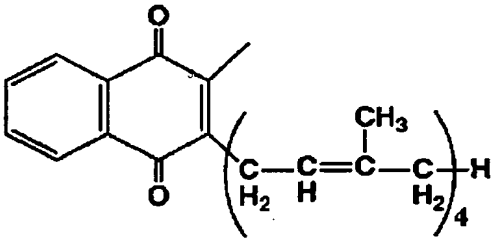
* NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to therapy / improvement agent of diseases, such as a hematopoietic organ neoplasm, a solid neoplasm, etc. based on a cell differentiation induction (following, differentiation inducing) operation.
[0002]
[Background of the Invention] It occupies [cancer / come] the primacy of the cause of death in our country and is long, and moreover, the number of patients is increasing every year and, now, development of a drugs and a cure with high effectiveness and safety serves as the maximum concerns of people, a researcher, and administration.
[0003] Although cancer (neoplasm) is variably classified according to a manifestation part, a pathology image, a symptom, etc., the leukemia which is the typical disease of a hematopoietic organ neoplasm is the neoplasm of a blood cell (leucocyte), and growth of various undifferentiated juvenile form leucocyte cells is the description. Moreover, the many can be divided roughly into the symptom based on control of normal hemopoiesis, and the symptom based on the infiltration and pressure to other organs although the clinical manifestations in the tumor cell which is increasing has classified into with chronic leukemia what is acute leukemia and a mature cell about what is an immature blast cell, and various also in them are presented. Reduction of a normal corpuscle cell appears as the infectious disease by the ischemia and agranulocytosis by the hypoglobulia, or a bleeding tendency by reduction of generation of heat and a platelet, and, specifically, control of normal hemopoiesis causes bone marrow incompetence. Generally it was just going to be known well that cancer is a disease with a poor prognosis, and the various drugs and therapy approaches have so far been examined.
[0004] In order to say that the view which serves as a foundation of a drug cure also in them acquires a curative effect by annihilating all the leukemic cells that are tumor cells and to improve [therefore] better treatment results, development of the drugs which proliferation potential shows the killer cell operation by cytotoxicity more powerfully to an unusually high neoplasm, a combination therapy, high concentration, an abundant administration therapy, etc. have been tried. However, in order that these drugs and cures might show toxicity also to a normal cell rather than may act only on a tumor cell specifically, critical side effects, such as the heart and a myocardiopathy, bone-marrow-activity control, nausea and vomiting, neuropathy, and depilation, were discovered, and there was a limitation also in a curative effect.
[0005] Although the fact that the various higher differentiaion inducers of safety carry out differentiation inducing of the tumor cell to a mature cell in in vitro is known on the other hand as compared with the conventional anticancer agent and the expectations for a differentiation derivation therapy had gathered, though it was regrettable, clinical usefulness was not accepted with the conventional differentiaion inducer. 1988 [however,] — Hyunh (Huang) ** — an all transformer-retinoic acid The clinical results which (the following and ATRA) brought the complete cure near 100% to the before [acute] myelogenous leukemia (following, APL) patient are reported. Since then [brad (Blood), The effectiveness is reconfirmed in 72,567-572, 1988.], and every country in the world, and expectation is growing in the differentiation derivation therapy over the cancer of the large range not only including a hematopoietic organ neoplasm but a solid neoplasm.
[0006]
[Description of the Prior Art] As mentioned above, ATRA sets to clinical. That it is effective in APL Hyunh and others (Huang) [a brad (Blood), 72,567-572, 1988.] is begun. KYASUTEN et al. (Castaigne) [a brad (Blood), 76, 1704-1709, 1990.], WARERU (Warrell) ** — [— the researcher has reported many New England journal OBU

Mehdi Soon (New Engl.J.Med.), 324, 1385-1393, 1991.], etc.
[0007] Moreover, Olson and others (Olsson) is vitamin D3. It is bioactive mold metabolite. 1alpha and 25-dihydroxycholecalciferol (following and activity V.D3) are Homo sapiens lymphoma culture cell lineage (U937). It has reported setting and having a differentiation-inducing operation [a cancer research (Cancer Res.), 43 (12Pt1), 5862-5867, 1983.]. development of activity V.D3 derivative which has a differentiation-inducing operation from this is also performed briskly — having — coming — JP,61-33165,A — 24-alkyl DEHIDORO vitamin D3 a derivative has antitumor action — moreover, Provisional Publication No. It is indicated, respectively that a 20-OKISA-21-NORU-vitamin-D3 derivative has a differentiation-inducing operation in 61 No. -140560 official report.
[0008] Tsang (Zhang) ** — HL60 and U937 whose bufalin (Bufalin) is the culture cell lineage of a Homo sapiens leukemic cell — and — [Biochemical - and - biotechnology physical Research Communications (Biochem.Biophys.Res.Commun.), 178 (2), 686-693, 1991. and the cancer research (Cancer Res.), 52 (17), 4634-4641, 1992.] which have reported that the differentiation-inducing operation was shown in ML1 .
[0009] As a compound which has a differentiation-inducing operation besides the above, foolish RANI and others (Baccarani) cytosine arabinoside (Ara-C) Moreover, [British journal OBU hematology (Br.J.Haematol.), 42,485-487, 1979.], Maureen (Morin) ** — aclacinomycin A — [a cancer research (Cancer Res.), 44, 2807-2812, 1984.] Moriya and others has reported interferon alpha to [clinical blood, 32,170-172, 1991.].
[0010] Ishikura and others is [the ROIKEMIA research (Leukemia Res.), 8 (5), 843-852, 1984.] which have reported that a geranyl farnesol (3, 7, 11, 15, 19-pentamethyl - 2, 6, 10, 14, 18-eicosa pen TAEN-1-oar) has a differentiation-inducing operation using the culture cell lineage of the mouse myeloleukemia. .
[0011]
[Problem(s) to be Solved by the Invention] Although ATRA and its derivative are used for the therapy of the psoriasis which is skin carcinoma and an intractable skin keratinization disease It is known widely that it will be easy to discover overvitamin A symptoms, such as hypertrophy, a neurological disorder, anorexia, vomiting, depilation, a feeling of the pruritus, etc. of liver, if a medicine is prescribed for the patient for a long period of time since lipophilicity is very high. And even if it stops administration, in order to remain in liver or an organization for a long period of time, once a side effect is discovered, there is a serious fault which does not disappear for a long period of time. Moreover, ATRA Although it was as above-mentioned that it is effective in APL, there were very few rates that APL occupies in [all] a leukemia patient as about 5%, and they were invalids at the acute leukemia patient of many other types. [most] There was also a problem which will be easy to recur if after remission furthermore stops administration.
[0012] Vitamin D3 Although the derivative is used for the therapy of osteoporosis etc., since the calcium absorption by the intestinal tract and the calcium resorption in the kidney are promoted, if a dose becomes superfluous, a hypercalcemia will be caused and bringing about the kidney trouble resulting from mineralization and a digestive organ failure is known. For this reason, a serum calcium value must be periodically inspected during an administration period, and it has in clinical the trouble which is very hard to use. Furthermore, it is vitamin D3. Although the differentiation-inducing operation of a derivative is effective in HL60 which is the culture cell lineage of Homo sapiens promyelocyte leukemia, effectiveness is not accepted in the model of other types.
[0013] Since the bufalin was not applied to clinical, about the safety, it is completely unknown and was not able to predict usefulness in Homo sapiens.
[0014] It was not like [from which neither cytosine arabinoside nor aclacinomycin A was furthermore also permitted to as drugs at home from the problem on safety, but the antitumor action of interferon alpha was also expected].
[0015] The evaluation result about a differentiation-inducing operation of a geranyl farnesol can be set to mouse leukemia cell culture cell lineage. Since the evaluation result in Homo sapiens leukemia cell culture cell lineage was not reported at all after that, when the drug sensitivity difference between the cells from which a seed differs was taken into consideration, the effectiveness in Homo sapiens was unknown entirely.
[0016] Thus, the present condition is that there are no drugs which combine the effectiveness which was excellent to various cancers, and safety, and development of the high drugs of usefulness was strongly desired to wide range cancer by clinical.
[0017]
[Means for Solving the Problem] The menatetrenone (vitamin K 2) concerning this invention is a vitamin K as a hemostasis vitamin. It is well-known as a compound which has an improvement operation of the following

disease by lack, and a symptom, and is widely used by clinical as drugs.
(1) Hypoprothrombinemia by biliary obstruction and the bile hyposecretion (2) Newborn infant hypoprothrombinemia (3) Intrapartum hemorrhage (4) Hypoprothrombinemia which happens during coumarin system anticoagulant administration [0018] There are still few own side effects of menatetrenone, and it is also a compound with very high safety. this invention persons have also examined the effectiveness to a disease besides many years paying attention to menatetrenone having the requirements that the safety to the great bioactive and Homo sapiens, or an animal is high. Consequently, also unexpectedly menatetrenone also had the differentiation-inducing operation, it found out that the desired end could be attained as a therapy / improvement agent to various cancers, such as a hematopoietic organ neoplasm and a solid neoplasm, and this invention was completed. Menatetrenone is expressed with the following chemical structure type.
[0019]
[Formula 2]



[0020] Although had 4 sets of double bonds in intramolecular, a total of eight kinds of geometrical isomers existed two kinds at a time (E bodies, Z body) about 3 sets among those, menatetrenone is set to this invention, the isomer of a gap is sufficient and it is not limited, an all transformer object is more desirable. Moreover, in this invention, one kind in these geometrical isomers may be used independently, two or more kinds of mixture may be used, and it is not limited. The origin is not limited, either, although there are furthermore a natural extract and synthetic compounds in menatetrenone. In addition, menatetrenone is widely sold as drugs, cosmetics, food, an industrial raw material, etc., and can come to hand easily.
[0021] Therefore, the purpose of this invention is to offer therapy / improvement agent to various cancers with the high clinical usefulness which has a differentiation-inducing operation. It is related with therapy / improvement agent of various cancer and malignant tumors, such as a hematopoietic organ neoplasm, a solid neoplasm, etc. which specifically makes menatetrenone an active principle, and therapy / improvement agent of a disease with an effective differentiation-inducing operation of this compound. Here as an example of the concrete disease name of a hematopoietic organ neoplasm Acute leukemia, chronic leukemia, a malignant lymphoma, a multiple myeloma, a macroglobulinemia, etc. can be mentioned. For example, as a solid neoplasm For example, a brain tumor, a head and neck cancer, a breast cancer, lung cancer, an esophagus cancer, gastric cancer, colon cancer, hepatic carcinoma, A gallbladder and a cholangioma, a pancreatic cancer, islet cell cancer, renal cell carcinoma, adrenal cortical adenocarcinoma, vesical cancer, Although a prostatic cancer, the orchioncus, an ovarian cancer, a uterine cancer, a choriocarcinoma, a thyroid cancer, a carcinoid-type-bronchial-adenoma neoplasm, skin carcinoma, a malignant melanoma, an osteosarcoma, a soft tissue sarcoma, a neuroblastoma, a Wilms' tumor, the embryonal rhabdomyosarcomas, a retinoblast kind, etc. can be mentioned It cannot be overemphasized that the object disease of this invention is not limited to these.
[0022] Moreover, since very high safety is expectable even if it prescribes a medicine for the patient in this invention for a long period of time in addition to the effectiveness as the above-mentioned therapy / improvement agent, it becomes possible to continue a prolonged therapy and it can be said that it is invention which contributes to the improvement of a cancer patient's quality of life greatly.
[0023] Next, in order to show the safety of menatetrenone, an acute toxicity test test result (fifty percent lethal dose value) is shown.
[Table 1]

メナテトレノンの急性毒性 (mg/Kg)

動物種	性別	経口	皮下	腹腔内
マウス (ICR系)	♂	> 5,000	> 5,000	> 5,000
	♀	> 5,000	> 5,000	> 5,000
ラット (SD系)	♂	> 5,000	> 5,000	> 5,000
	♀	> 5,000	> 5,000	> 5,000

[0024] The very high safety of menatetrenone is clear from Table 1. Menatetrenone is already used by clinical widely as drugs still as mentioned above, and the safety is checked.
[0025] As an administration pharmaceutical form, oral pharmaceutical preparation, such as powder, a fine grain agent, a granule, a tablet, a covering tablet, and a capsule, injection pharmaceutical preparation, and external preparations (endermic pharmaceutical preparation) are mentioned, for example. In the case of pharmaceutical-preparation-izing, it can manufacture with a conventional method using the usual pharmaceutical preparation support.
[0026] That is, in order to manufacture oral pharmaceutical preparation, menatetrenone, an excipient, and after adding a binder, disintegrator, lubricant, a coloring agent, correctives, etc. if needed further, it considers as powder, a fine grain agent, a granule, a tablet, a covering tablet, a capsule, etc. with a conventional method.
[0027] As an excipient, a lactose, corn starch, white soft sugar, grape sugar, a mannitol, a sorbitol, crystalline cellulose, a silicon dioxide, etc., for example as a binder For example, polyvinyl alcohol, polyvinyl ether, methyl cellulose, Ethyl cellulose, gum arabic, tragacanth, gelatin, a shellac, The hydroxypropyl methylcellulose, hydroxypropylcellulose, A polyvinyl pyrrolidone, polypropylene-glycol polyoxyethylene block polymer, meglumine, etc. as disintegrator For example, starch, an agar, the end of gelatin, crystalline cellulose, a calcium carbonate, A sodium hydrogencarbonate, calcium citrate, a dextrin, pectin, carboxymethyl-cellulose calcium, etc. as lubricant For example, what is permitted that magnesium stearate, talc, a polyethylene glycol, a silica, hardening vegetable oil, etc. add in drugs as a coloring agent is used for the menthol, aromatic powder, mentha oil, camphor Borneo, a cinnamomi cortex pulveratus, etc. as correctives in the end of cocoa. Of course, these tablets and granules are not hindered by coating suitably according to glycocalyx and other need.
[0028] Moreover, in case the pharmaceutical preparation for injection is manufactured, a solubilizing agent, a stabilizing agent, etc. are added to menatetrenone pH regulator, a resolvent, an isotonizing agent, etc. and if needed, and it pharmaceutical-preparation-izes with a conventional method.
[0029] The approach at the time of manufacturing external preparations is not limited, but can be manufactured with a conventional method. That is, it is possible to use the various raw materials usually used for drugs, quasi drugs, cosmetics, etc. as a base ingredient used in pharmaceutical-preparation-izing.
[0030] As a base ingredient to be used, specifically For example, animal and vegetable oils, straight mineral oil, ester oil, Waxes, higher alcohol, fatty acids, silicone oil, a surfactant, Although raw materials, such as phospholipid, alcohols, polyhydric alcohol, water soluble polymers, clay minerals, and purified water, are mentioned and pH regulator, an anti-oxidant, a chelating agent, a preservation-from-decay antifungal agent, a coloring agent, perfume, etc. can be added further if needed The base ingredient of the external preparations concerning this invention is not limited to these. Moreover, components, such as the component which has other differentiation-inducing operations if needed, a blood-flow accelerator, a germicide, an antiphlogistic, a cell activator, vitamins, amino acid, a moisturizer, and a keratolytic drug, can also be blended. In addition, the addition of the above-mentioned base ingredient is an amount which becomes the concentration usually set up in manufacture of external preparations.
[0031] although the clinical doses of the menatetrenone in this invention differ, and are not limited by a symptom, severity, age, complication, etc. and it changes with the class, routes of administration, etc. of a compound — usually — an adult — 10mg - 10g per day it is — desirable — 50mg - 5g — it is — further — desirable — 100mg - 1g — it is — this — the inside of taking orally and a vein — or dermal administration is carried out.
[0032] Next, although an example is hung up over below in order to explain this invention concretely, it cannot be overemphasized that this invention is not limited to these.
[0033]
[Example]
Example 1 Granule [0034]
[Table 2]

<処方>

	原料	配合量（mg）
1)	メナテトレノン	100.0
2)	無水ケイ酸	100.0
3)	D-マンニトール	450.0
4)	ヒドロキシプロピルセルロース	40.0
5)	dl-α-トコフェロール	0.2
6)	タルク	10.0
7)	乳糖	約 300.0

[0035] Example 2 Tablet [0036]
[Table 3]
<処方>

	原料	配合量（mg）
1)	メナテトレノン	10.0
2)	ヒドロキシプロピルセルロース	50.0
3)	乳糖	100.0
4)	トウモロコシデンプン	20.0
5)	無水ケイ酸	3.0
6)	ステアリン酸マグネシウム	0.2
7)	マクロゴール6000	3.0
8)	ポリビニルピロリドン	0.6
9)	アラビアゴム末	3.0
10)	沈降炭酸カルシウム	4.0
11)	酸化チタン	10.0
12)	タルク	15.0
13)	白糖	約 60.0

[0037] Example 3 Injections [0038]
[Table 4]
<処方>

	原料	配合量（重量%）
1)	メナテトレノン	1.0
2)	ポリオキシエチレンソルビタンモノオレート	3.5
3)	D-ソルビトール	5.0
4)	リン酸二水素ナトリウム（NaH_2PO_4）	0.08
5)	リン酸水素ナトリウム（Na_2HPO_4）	0.07
6)	精製水	加えて100.0

[0039] Example 4 External preparations [0040]
[Table 5]
<処方>

	原料	配合量（重量%）
1)	メナテトレノン	1.0
2)	スクワラン	10.0
3)	ミリスチン酸イソプロピル	7.0
4)	ベヘニルアルコール	1.0
5)	セトステアリルアルコール	5.5
6)	ステアリン酸モノグリセリン	2.0
7)	d-α-トコフェロール	0.05
8)	POE（20）モノステアリン酸ソルビタン	2.0
9)	キサンタンガム	0.1
10)	1、3-ブチレングリコール	2.0
11)	グリセリン	3.0
12)	D-ソルビトール	5.0
13)	パラベン	0.2
14)	精製水	加えて100.0

[0041]
[Effect of the Invention] Next, in order to show the usefulness as a differentiaion inducer of this invention compound, it is a mouse B16. The example of an effectiveness experiment over a melanoma cell and various

Homo sapiens leukemia culture cell lineage is given. In addition, the Homo sapiens leukemia culture cell lineage used for the experiment is as follows.
(1) HL60; before [Homo sapiens] myelogenous leukemia cell (2) U937; Homo sapiens monoblast Mr. leukemic cell (3) ML1 ; Homo sapiens myeloblast Mr. leukemic cell (4) K562; Homo sapiens bone marrow erythroblast leukemic cell [0042] Experiment 1 Mouse B16 The differentiation-inducing operation (approach) mouse origin of menatetrenone to a melanoma cell Melanin generation ability was evaluated for the differentiation-inducing operation of the menatetrenone to B16 melanoma cell as an index. That is, it is B16 melanoma cell The subculture back and 2x104 A cel/ml It becomes and needs. In addition to 10%FCS MEM*, it cultivated with the petri dish (phi= 10cm) for culture for 24 hours. Each sample prepared after culture to the concentration (1.0x10-6 M) which did not show toxicity. 10%FCS MEM On these conditions after performing culture-medium exchange It cultivated for five days. An isotonicity balanced salt solution [NISSUI PHARMACEUTICAL make and trade name;Dulbecco's PBS (-)] washes after culture, and it is 0.25%. After it collected cells using the trypsin / ethylene-diamine-tetraacetic acid (EDTA) solution and the above-mentioned isotonicity balanced salt solution washed again further, centrifugal separation (100G) was carried out and the cell was obtained. (10%FCS MEM*; culture medium which added foetal calf serum, penicillin, streptomycin, and a sodium hydrogencarbonate 10% to the standard culture medium)
[0043] the obtained cell — a 1mM-phenylmethyl sulfonyl fluoride (PMSF) — after adding the phosphate buffer solution which added 1ml, according to Oikawa's and others approach (Eire Journal of Biological Medicine [Yale J.Biol.Med.], 46,500-507, 1973.), the absorbance (lambda= 400nm) measured and estimated the total amount of melanin.
[0044] To Table 6, it is the mouse origin. A differentiation-inducing operation of the menatetrenone to B16 melanoma cell is shown.
[0045]
[Table 6]
マウスＢ１６メラノーマ細胞に対するメナテトレノンの分化誘導作用

試　料	培養細胞タンパク量あたりの総メラニン量（%）
メナテトレノン	８５
コントロール	１００

[0046] as [be / clear from Table 6] — 1.0x10-6M Culture processing was carried out for five days in menatetrenone. the total amount of melanin per amount of proteins of B16 melanoma cell (you melanin and phaeomelanin) — a control cultured cell — comparing — abbreviation It fell 15%. Having decreased clearly by menatetrenone processing the amount of intracellular tyrosinases at this time It was checked by the SDS electrophoresis method.
[0047] The above-mentioned result does not show the effectiveness over the solid neoplasm of menatetrenone, and suggests the broad adaptability which does not stop only at differentiation inducing of the hematopoietic organ neoplasm of menatetrenone.
[0048] Experiments 2-5 Evaluation of the differentiation-inducing operation concerning the differentiation-inducing operation (approach) experiments 2-5 of the menatetrenone to various Homo sapiens leukemia culture cell lineage is an approach [Nakatani et al., a cancer research (Cancer Res.), 48, 4201-4205, 1988.] indicated by reference. It carried out by having followed, and measured and evaluated about the following differentiation-inducing marker.
(1) The nitroblue tetrazolium (following and NBT) reduction ability which is a differentiation-inducing marker to a normal cell is a cell. The microscope observed and estimated the formazan which incubated for 40 minutes, and it was returned and was produced at an NBT reagent and 37 degrees C.
(2) The cell of a dead cell dyed comparatively (viability of a cell) with the trypan blue reagent was used as the dead cell, and the percentage to the whole number of cells was computed.
[0049] (Result)
experiment 2 Before [Homo sapiens] myelogenous leukemia cell HL60 the receiving differentiation-inducing operation — a degree — Homo sapiens myeloblast Mr. leukemic cell HL60 The receiving concentration of menatetrenone and the relation of a differentiation-inducing operation are shown in drawing 1 .
[0050]
[Drawing 1]
[0051] clear from drawing 1 — as — increment in menatetrenone concentration NBT reduction ability

(differentiation induction potency) increases — 1.0x10-5M menatetrenone processing — abbreviation Differentiation was accepted in 57% of cell. on the other hand — the number of cells — the increment in menatetrenone concentration — decreasing — the same — 1.0x10-5M Abbreviation of control by menatetrenone processing It decreased to 48% and growth inhibitory action was also accepted. the increment in the number of dead cells, and 1.0x10-5M up to — as compared with control, it came out only, and it is and most cytotoxicity was not accepted. Therefore, it is clear menatetrenone's to guide differentiation of HL60 cell characteristic, without being based on cytotoxicity.

[0052] Experiment 3 Homo sapiens monoblast Mr. leukemic cell U937 Receiving differentiation-inducing operation Homo sapiens monoblast Mr. leukemic cell U937 The receiving menatetrenone concentration and the relation of a differentiation-inducing operation are shown in drawing 2 .

[0053]

[Drawing 2]

[0054] clear from drawing 2 — as — increment in menatetrenone concentration NBT reduction ability (differentiation induction potency) increases — 1.0x10-6M menatetrenone processing — abbreviation Differentiation was accepted in 84% of cell. on the other hand — the number of cells — the increment in menatetrenone concentration — decreasing — the same — 1.0x10-6M Abbreviation of control by menatetrenone processing It became 61% and growth inhibitory action was also accepted. the number of dead cells, and 5.0x10-6M up to — the difference was not accepted as compared with control and cytotoxicity was not accepted. Therefore, it is clear menatetrenone's to guide differentiation of U937 cell characteristic, without being based on cytotoxicity.

[0055] Experiment 4 Homo sapiens myeloblast Mr. leukemic cell Differentiation-inducing operation Homo sapiens myeloblast Mr. leukemic cell to ML1 The menatetrenone concentration to ML1 and the relation of a differentiation-inducing operation are shown in drawing 3 .

[0056]

[Drawing 3]

[0057] clear from drawing 3 — as — increment in menatetrenone concentration NBT reduction ability (differentiation induction potency) increases — 1.0x10-6M menatetrenone processing — abbreviation Differentiation was accepted in 84% of cell. on the other hand — the number of cells — the increment in menatetrenone concentration — decreasing — the same — 1.0x10-6M Abbreviation of control by menatetrenone processing It became 61% and growth inhibitory action was also accepted. the number of dead cells, and 1.0x10-6M up to — the difference was not accepted as compared with control and cytotoxicity was not accepted. Therefore, menatetrenone is on the description target, without being based on cytotoxicity. It is clear to guide differentiation of ML1 cell.

[0058] Experiment 5 Homo sapiens bone marrow erythroblast leukemic cell K562 Receiving differentiation-inducing operation Homo sapiens bone marrow erythroblast leukemic cell K562 The receiving menatetrenone concentration and the relation of a differentiation-inducing operation are shown in drawing 4 .

[0059]

[Drawing 4]

[0060] clear from drawing 4 — as — increment in menatetrenone concentration NBT reduction ability (differentiation induction potency) increases clearly — 1.0x10-6M Menatetrenone processing Differentiation was accepted in 60% of cell. On the other hand, it is the number of cells. 1.0x10-5M It decreased in the above high concentration and cell proliferation depressant action was weak. the number of dead cells, and 2.0x10-5M up to — the difference was not accepted as compared with control and cytotoxicity was not accepted. Although menatetrenone is not so stronger as it receives each cell of HL60, U937, and ML1 as mentioned above than this result, it is clear to also guide differentiation of Homo sapiens bone marrow erythroblast leukemic cell K562 cell.

[0061] The result of the above-mentioned example of an experiment to menatetrenone is 10-6-10-5M. In concentration, it is clear to guide differentiation of the various Homo sapiens leukemic cells from which a developmental stage differs. And it divides and they are a myeloblast Mr. leukemic cell (HL60), a monoblast Mr. leukemic cell (U937), and a myeloblast Mr. leukemic cell (ML1). It is characteristic that the effectiveness of receiving is more remarkable, and it also has the differentiation induction potency to a bone marrow erythroblast leukemic cell.

[0062] Experiment 6 To the effectiveness pan in various Homo sapiens leukemia culture cell lineage which menatetrenone gives to other differentiation-inducing markers In addition to NBT reduction ability and the number of cells, the following markers are used as an index of differentiation inducing, and it has the respectively

following semantics.
(1) AS-D-chloro acetate esterase activity; differentiation to granulocyte (2) alpha-naphthyl acetate esterase activity; differentiation to monocyte (macrophage) (3) Phagocytic activity; differentiation to a normal leucocyte cell (4) The number of Fc receptors; differentiation to granulocyte and monocyte [0063] (Approach) For measurement of the above-mentioned marker, (1) and (2) are said reference [Nakatani et al., a cancer research (Cancer Res.), 48, 4201-4205, 1988.]. The approach of a publication was followed. (3) **, the cell, and the polystyrene latex bead were incubated at 37 degrees C for 4 hours, and the number of cells which incorporated ten or more beads was counted. Cell viability (viable cell comparatively) made the viable cell the cell which is not dyed with a trypan blue reagent, and computed the percentage to the whole number of cells as phagocytic activity. (4) ** molecular immunology (Molecular Immunology), 25 (11), 1159-67, and 1988. The indicated approach was followed.
[0064] (Result) Menatetrenone shows below the effectiveness given to the above-mentioned differentiation-inducing marker in Table 7.
[0065]
[Table 7]
その他の分化誘導マーカーに対するメナテトレノンの効果

細胞	メナテトレノン濃度 (M)	ASD-クロロアセテートエステラーゼ活性	α-ナフチルアセチルエステラーゼ活性	貪食能	Fc レセプター数
HL60	コントロール	36.5± 5.6	12.6± 1.8	12.8± 3.1	1.6± 0.2
	1×10^{-6}	37.5± 2.0	15.3± 2.2	21.4± 4.4	1.8± 0.1
	2×10^{-6}	27.6± 3.4	22.7± 3.6	37.5±13.5	1.7± 0.1
U937	コントロール	7.2± 2.2	6.2± 1.7	12.5± 2.9	1.2± 0.3
	1×10^{-6}	13.2± 0.2	13.9± 4.2	17.8± 1.2	12.5±10.7
	2×10^{-6}	10.0± 4.2	33.1±10.3	18.2± 6.7	8.8± 2.7
ML1	コントロール	11.3± 4.2	9.7± 2.3	8.9± 3.5	2.5± 0.9
	2×10^{-6}	27.8± 2.6	14.3± 3.2	34.4±12.9	31.4± 5.0
	1×10^{-5}	38.6± 7.4	34.8±14.4	40.0± 0.0	13.0± 0.0
K562	コントロール	6.2± 0.4	5.3± 2.5	15.2± 7.9	2.8± 2.0
	1×10^{-6}	6.7± 3.8	2.6± 1.3	19.5± 3.0	3.6± 1.9
	2×10^{-6}	6.9± 3.2	4.0± 0.9	10.2± 3.0	6.7± 3.2

[0066] It is a Homo sapiens myeloblast Mr. leukemic cell by menatetrenone processing so that clearly from Table 7. HL60 And Homo sapiens monoblast Mr. leukemic cell U937 It turns out that it set, alpha-naphthyl acetate esterase activity and phagocytic activity were normalized, and differentiation inducing of these Homo sapiens leukemic cells was carried out to monocyte (macrophage). Moreover, Homo sapiens myeloblast Mr. leukemic cell In ML1, it turns out that all differentiation-inducing markers are improved and differentiation inducing was carried out to granulocyte and monocyte. It is clearer than these results menatetrenone's to have differentiation induction potency widely to the various Homo sapiens leukemia cultured cells from which a type differs.
[0067]

[Translation done.]

* NOTICES *

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TECHNICAL FIELD

[Industrial Application] This invention relates to therapy / improvement agent of diseases, such as a hematopoietic organ neoplasm, a solid neoplasm, etc. based on a cell differentiation induction (following, differentiation inducing) operation.
[0002]
[Background of the Invention] It occupies [cancer / come] the primacy of the cause of death in our country and is long, and moreover, the number of patients is increasing every year and, now, development of a drugs and a cure with high effectiveness and safety serves as the maximum concerns of people, a researcher, and administration.
[0003] Although cancer (neoplasm) is variably classified according to a manifestation part, a pathology image, a symptom, etc., the leukemia which is the typical disease of a hematopoietic organ neoplasm is the neoplasm of a blood cell (leucocyte), and growth of various undifferentiated juvenile form leucocyte cells is the description. Moreover, the many can be divided roughly into the symptom based on control of normal hemopoiesis, and the symptom based on the infiltration and pressure to other organs although the clinical manifestations in the tumor cell which is increasing has classified into with chronic leukemia what is acute leukemia and a mature cell about what is an immature blast cell, and various also in them are presented. Reduction of a normal corpuscle cell appears as the infectious disease by the ischemia and agranulocytosis by the hypoglobulia, or a bleeding tendency by reduction of generation of heat and a platelet, and, specifically, control of normal hemopoiesis causes bone marrow incompetence. Generally it was just going to be known well that cancer is a disease with a poor prognosis, and the various drugs and therapy approaches have so far been examined.
[0004] In order to say that the view which serves as a foundation of a drug cure also in them acquires a curative effect by annihilating all the leukemic cells that are tumor cells and to improve [therefore] better treatment results, development of the drugs which proliferation potential shows the killer cell operation by cytotoxicity more powerfully to an unusually high neoplasm, a combination therapy, high concentration, an abundant administration therapy, etc. have been tried. However, in order that these drugs and cures might show toxicity also to a normal cell rather than may act only on a tumor cell specifically, critical side effects, such as the heart and a myocardiopathy, bone-marrow-activity control, nausea and vomiting, neuropathy, and depilation, were discovered, and there was a limitation also in a curative effect.
[0005] Although the fact that the various higher differentiaion inducers of safety carry out differentiation inducing of the tumor cell to a mature cell in in vitro is known on the other hand as compared with the conventional anticancer agent and the expectations for a differentiation derivation therapy had gathered, though it was regrettable, clinical usefulness was not accepted with the conventional differentiaion inducer. 1988 [however,] — Hyunh (Huang) ** — an all transformer-retinoic acid The clinical results which (the following and ATRA) brought the complete cure near 100% to the before [acute] myelogenous leukemia (following, APL) patient are reported. Since then [brad (Blood), The effectiveness is reconfirmed in 72,567-572, 1988.], and every country in the world, and expectation is growing in the differentiation derivation therapy over the cancer of the large range not only including a hematopoietic organ neoplasm but a solid neoplasm.

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PRIOR ART

[Description of the Prior Art] As mentioned above, ATRA sets to clinical. That it is effective in APL Hyunh and others (Huang) [a brad (Blood), 72,567-572, 1988.] is begun. KYASUTEN et al. (Castaigne) [a brad (Blood), 76, 1704-1709, 1990.], WARERU (Warrell) ** -- [-- the researcher has reported many New England journal OBU Mehdi Soon (New Engl.J.Med.), 324, 1385-1393, 1991.], etc.
[0007] Moreover, Olson and others (Olsson) is vitamin D3. It is bioactive mold metabolite. 1alpha and 25-dihydroxycholecalciferol (following and activity V.D3) are Homo sapiens lymphoma culture cell lineage (U937). It has reported setting and having a differentiation-inducing operation [a cancer research (Cancer Res.), 43 (12Pt1), 5862-5867, 1983.]. development of activity V.D3 derivative which has a differentiation-inducing operation from this is also performed briskly -- having -- coming -- JP,61-33165,A -- 24-alkyl DEHIDORO vitamin D3 a derivative has antitumor action -- moreover, Provisional Publication No. It is indicated, respectively that a 20-OKISA-21-NORU-vitamin-D3 derivative has a differentiation-inducing operation in 61 No. -140560 official report.
[0008] Tsang (Zhang) ** -- HL60 and U937 whose bufalin (Bufalin) is the culture cell lineage of a Homo sapiens leukemic cell -- and -- [Biochemical - and - biotechnology physical Research Communications (Biochem.Biophys.Res.Commun.), 178 (2), 686-693, 1991. and the cancer research (Cancer Res.), 52 (17), 4634-4641, 1992.] which have reported that the differentiation-inducing operation was shown in ML1 .
[0009] As a compound which has a differentiation-inducing operation besides the above, foolish RANI and others (Baccarani) cytosine arabinoside (Ara-C) Moreover, [British journal OBU hematology (Br.J.Haematol.), 42,485-487, 1979.], Maureen (Morin) ** -- aclacinomycin A -- [a cancer research (Cancer Res.), 44, 2807-2812, 1984.] Moriya and others has reported interferon alpha to [clinical blood, 32,170-172, 1991.].
[0010] Ishikura and others is [the ROIKEMIA research (Leukemia Res.), 8 (5), 843-852, 1984.] which have reported that a geranyl farnesol (3, 7, 11, 15, 19-pentamethyl - 2, 6, 10, 14, 18-eicosa pen TAEN-1-oar) has a differentiation-inducing operation using the culture cell lineage of the mouse myeloleukemia. .

[Translation done.]

EFFECT OF THE INVENTION

[Effect of the Invention] Next, in order to show the usefulness as a differentiaion inducer of this invention compound, it is a mouse B16. The example of an effectiveness experiment over a melanoma cell and various Homo sapiens leukemia culture cell lineage is given. In addition, the Homo sapiens leukemia culture cell lineage used for the experiment is as follows.
(1) HL60; before [Homo sapiens] myelogenous leukemia cell (2) U937; Homo sapiens monoblast Mr. leukemic cell (3) ML1 ; Homo sapiens myeloblast Mr. leukemic cell (4) K562; Homo sapiens bone marrow erythroblast leukemic cell [0042] Experiment 1 Mouse B16 The differentiation-inducing operation (approach) mouse origin of menatetrenone to a melanoma cell Melanin generation ability was evaluated for the differentiation-inducing operation of the menatetrenone to B16 melanoma cell as an index. That is, it is B16 melanoma cell The subculture back and 2x104 A cel/ml It becomes and needs. In addition to 10%FCS MEM*, it cultivated with the petri dish (phi= 10cm) for culture for 24 hours. Each sample prepared after culture to the concentration (1.0x10-6 M) which did not show toxicity. 10%FCS MEM On these conditions after performing culture-medium exchange It cultivated for five days. An isotonicity balanced salt solution [NISSUI PHARMACEUTICAL make and trade name;Dulbecco's PBS (-)] washes after culture, and it is 0.25%. After it collected cells using the trypsin / ethylene-diamine-tetraacetic acid (EDTA) solution and the above-mentioned isotonicity balanced salt solution washed again further, centrifugal separation (100G) was carried out and the cell was obtained. (10%FCS MEM*; culture medium which added foetal calf serum, penicillin, streptomycin, and a sodium hydrogencarbonate 10% to the standard culture medium)
[0043] the obtained cell — a 1mM-phenylmethyl sulfonyl fluoride (PMSF) — after adding the phosphate buffer solution which added 1ml, according to Oikawa's and others approach (Eire Journal of Biological Medicine [Yale J.Biol.Med.], 46,500-507, 1973.), the absorbance (lambda= 400nm) measured and estimated the total amount of melanin.
[0044] To Table 6, it is the mouse origin. A differentiation-inducing operation of the menatetrenone to B16 melanoma cell is shown.
[0045]
[Table 6]
マウスＢ１６メラノーマ細胞に対するメナテトレノンの分化誘導作用

試　料	培養細胞タンパク量あたりの総メラニン量（%）
メナテトレノン	８５
コントロール	１００

[0046] as [be / clear from Table 6] — 1.0x10-6M Culture processing was carried out for five days in menatetrenone. the total amount of melanin per amount of proteins of B16 melanoma cell (you melanin and phaeomelanin) — a control cultured cell — comparing — abbreviation It fell 15%. Having decreased clearly by menatetrenone processing the amount of intracellular tyrosinases at this time It was checked by the SDS electrophoresis method.
[0047] The above-mentioned result does not show the effectiveness over the solid neoplasm of menatetrenone, and suggests the broad adaptability which does not stop only at differentiation inducing of the hematopoietic organ neoplasm of menatetrenone.
[0048] Experiments 2-5 Evaluation of the differentiation-inducing operation concerning the differentiation-inducing operation (approach) experiments 2-5 of the menatetrenone to various Homo sapiens leukemia culture cell lineage is an approach [Nakatani et al., a cancer research (Cancer Res.), 48, 4201-4205, 1988.] indicated by

reference. It carried out by having followed, and measured and evaluated about the following differentiation-inducing marker.
(1) The nitroblue tetrazolium (following and NBT) reduction ability which is a differentiation-inducing marker to a normal cell is a cell. The microscope observed and estimated the formazan which incubated for 40 minutes, and it was returned and was produced at an NBT reagent and 37 degrees C.
(2) The cell of a dead cell dyed comparatively (viability of a cell) with the trypan blue reagent was used as the dead cell, and the percentage to the whole number of cells was computed.
[0049] (Result)
experiment 2 Before [Homo sapiens] myelogenous leukemia cell HL60 the receiving differentiation-inducing operation — a degree — Homo sapiens myeloblast Mr. leukemic cell HL60 The receiving concentration of menatetrenone and the relation of a differentiation-inducing operation are shown in drawing 1 .
[0050]
[Drawing 1]
[0051] clear from drawing 1 — as — increment in menatetrenone concentration NBT reduction ability (differentiation induction potency) increases — 1.0x10-5M menatetrenone processing — abbreviation Differentiation was accepted in 57% of cell. on the other hand — the number of cells — the increment in menatetrenone concentration — decreasing — the same — 1.0x10-5M Abbreviation of control by menatetrenone processing It decreased to 48% and growth inhibitory action was also accepted. the increment in the number of dead cells, and 1.0x10-5M up to — as compared with control, it came out only, and it is and most cytotoxicity was not accepted. Therefore, it is clear menatetrenone's to guide differentiation of HL60 cell characteristic, without being based on cytotoxicity.
[0052] Experiment 3 Homo sapiens monoblast Mr. leukemic cell U937 Receiving differentiation-inducing operation Homo sapiens monoblast Mr. leukemic cell U937 The receiving menatetrenone concentration and the relation of a differentiation-inducing operation are shown in drawing 2 .
[0053]
[Drawing 2]
[0054] clear from drawing 2 — as — increment in menatetrenone concentration NBT reduction ability (differentiation induction potency) increases — 1.0x10-6M menatetrenone processing — abbreviation Differentiation was accepted in 84% of cell. on the other hand — the number of cells — the increment in menatetrenone concentration — decreasing — the same — 1.0x10-6M Abbreviation of control by menatetrenone processing It became 61% and growth inhibitory action was also accepted. the number of dead cells, and 5.0x10-6M up to — the difference was not accepted as compared with control and cytotoxicity was not accepted. Therefore, it is clear menatetrenone's to guide differentiation of U937 cell characteristic, without being based on cytotoxicity.
[0055] Experiment 4 Homo sapiens myeloblast Mr. leukemic cell Differentiation-inducing operation Homo sapiens myeloblast Mr. leukemic cell to ML1 The menatetrenone concentration to ML1 and the relation of a differentiation-inducing operation are shown in drawing 3 .
[0056]
[Drawing 3]
[0057] clear from drawing 3 — as — increment in menatetrenone concentration NBT reduction ability (differentiation induction potency) increases — 1.0x10-6M menatetrenone processing — abbreviation Differentiation was accepted in 84% of cell. on the other hand — the number of cells — the increment in menatetrenone concentration — decreasing — the same — 1.0x10-6M Abbreviation of control by menatetrenone processing It became 61% and growth inhibitory action was also accepted. the number of dead cells, and 1.0x10-6M up to — the difference was not accepted as compared with control and cytotoxicity was not accepted. Therefore, menatetrenone is on the description target, without being based on cytotoxicity. It is clear to guide differentiation of ML1 cell.
[0058] Experiment 5 Homo sapiens bone marrow erythroblast leukemic cell K562 Receiving differentiation-inducing operation Homo sapiens bone marrow erythroblast leukemic cell K562 The receiving menatetrenone concentration and the relation of a differentiation-inducing operation are shown in drawing 4 .
[0059]
[Drawing 4]
[0060] clear from drawing 4 — as — increment in menatetrenone concentration NBT reduction ability (differentiation induction potency) increases clearly — 1.0x10-6M Menatetrenone processing Differentiation was

accepted in 60% of cell. On the other hand, it is the number of cells. 1.0x10-5M It decreased in the above high concentration and cell proliferation depressant action was weak. the number of dead cells, and 2.0x10-5M up to — the difference was not accepted as compared with control and cytotoxicity was not accepted. Although menatetrenone is not so stronger as it receives each cell of HL60, U937, and ML1 as mentioned above than this result, it is clear to also guide differentiation of Homo sapiens bone marrow erythroblast leukemic cell K562 cell.
[0061] The result of the above-mentioned example of an experiment to menatetrenone is 10-6-10-5M. In concentration, it is clear to guide differentiation of the various Homo sapiens leukemic cells from which a developmental stage differs. And it divides and they are a myeloblast Mr. leukemic cell (HL60), a monoblast Mr. leukemic cell (U937), and a myeloblast Mr. leukemic cell (ML1). It is characteristic that the effectiveness of receiving is more remarkable, and it also has the differentiation induction potency to a bone marrow erythroblast leukemic cell.
[0062] Experiment 6 To the effectiveness pan in various Homo sapiens leukemia culture cell lineage which menatetrenone gives to other differentiation-inducing markers In addition to NBT reduction ability and the number of cells, the following markers are used as an index of differentiation inducing, and it has the respectively following semantics.
(1) AS-D-chloro acetate esterase activity; differentiation to granulocyte (2) alpha-naphthyl acetate esterase activity; differentiation to monocyte (macrophage) (3) Phagocytic activity; differentiation to a normal leucocyte cell (4) The number of Fc receptors; differentiation to granulocyte and monocyte [0063] (Approach) For measurement of the above-mentioned marker, (1) and (2) are said reference [Nakatani et al., a cancer research (Cancer Res.), 48, 4201-4205, 1988.]. The approach of a publication was followed. (3) **, the cell, and the polystyrene latex bead were incubated at 37 degrees C for 4 hours, and the number of cells which incorporated ten or more beads was counted. Cell viability (viable cell comparatively) made the viable cell the cell which is not dyed with a trypan blue reagent, and computed the percentage to the whole number of cells as phagocytic activity. (4) ** molecular immunology (Molecular Immunology), 25 (11), 1159-67, and 1988. The indicated approach was followed.
[0064] (Result) Menatetrenone shows below the effectiveness given to the above-mentioned differentiation-inducing marker in Table 7.
[0065]
[Table 7]
その他の分化誘導マーカーに対するメナテトレノンの効果

細胞	メナテトレノン濃度 (M)	ASD-クロロアセテートエステラーゼ活性	α-ナフチルアセチルエステラーゼ活性	貪食能	Fc レセプター数
HL60	コントロール	36.5± 5.6	12.6± 1.8	12.8± 3.1	1.6± 0.2
	1×10^{-6}	37.5± 2.0	15.3± 2.2	21.4± 4.4	1.8± 0.1
	2×10^{-6}	27.6± 3.4	22.7± 3.6	37.5±13.5	1.7± 0.1
U937	コントロール	7.2± 2.2	6.2± 1.7	12.5± 2.9	1.2± 0.3
	1×10^{-6}	13.2± 0.2	13.9± 4.2	17.8± 1.2	12.5±10.7
	2×10^{-6}	10.0± 4.2	33.1±10.3	18.2± 6.7	8.8± 2.7
ML1	コントロール	11.3± 4.2	9.7± 2.3	8.9± 3.5	2.5± 0.9
	2×10^{-6}	27.8± 2.6	14.3± 3.2	34.4±12.9	31.4± 5.0
	1×10^{-5}	38.6± 7.4	34.8±14.4	40.0± 0.0	13.0± 0.0
K562	コントロール	6.2± 0.4	5.3± 2.5	15.2± 7.9	2.8± 2.0
	1×10^{-6}	6.7± 3.8	2.6± 1.3	19.5± 3.0	3.6± 1.9
	2×10^{-6}	6.9± 3.2	4.0± 0.9	10.2± 3.0	6.7± 3.2

[0066] It is a Homo sapiens myeloblast Mr. leukemic cell by menatetrenone processing so that clearly from Table 7. HL60 And Homo sapiens monoblast Mr. leukemic cell U937 It turns out that it set, alpha-naphthyl acetate esterase activity and phagocytic activity were normalized, and differentiation inducing of these Homo sapiens leukemic cells was carried out to monocyte (macrophage). Moreover, Homo sapiens myeloblast Mr. leukemic cell In ML1, it turns out that all differentiation-inducing markers are improved and differentiation inducing was carried out to granulocyte and monocyte. It is clearer than these results menatetrenone's to have differentiation induction potency widely to the various Homo sapiens leukemia cultured cells from which a type differs.

[Translation done.]

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] Although ATRA and its derivative are used for the therapy of the psoriasis which is skin carcinoma and an intractable skin keratinization disease It is known widely that it will be easy to discover overvitamin A symptoms, such as hypertrophy, a neurological disorder, anorexia, vomiting, depilation, a feeling of the pruritus, etc. of liver, if a medicine is prescribed for the patient for a long period of time since lipophilicity is very high. And even if it stops administration, in order to remain in liver or an organization for a long period of time, once a side effect is discovered, there is a serious fault which does not disappear for a long period of time. Moreover, ATRA Although it was as above-mentioned that it is effective in APL, there were very few rates that APL occupies in [all] a leukemia patient as about 5%, and they were invalids at the acute leukemia patient of many other types. [most] There was also a problem which will be easy to recur if after remission furthermore stops administration.
[0012] Vitamin D3 Although the derivative is used for the therapy of osteoporosis etc., since the calcium absorption by the intestinal tract and the calcium resorption in the kidney are promoted, if a dose becomes superfluous, a hypercalcemia will be caused and bringing about the kidney trouble resulting from mineralization and a digestive organ failure is known. For this reason, a serum calcium value must be periodically inspected during an administration period, and it has in clinical the trouble which is very hard to use. Furthermore, it is vitamin D3. Although the differentiation-inducing operation of a derivative is effective in HL60 which is the culture cell lineage of Homo sapiens promyelocyte leukemia, effectiveness is not accepted in the model of other types.
[0013] Since the bufalin was not applied to clinical, about the safety, it is completely unknown and was not able to predict usefulness in Homo sapiens.
[0014] It was not like [from which neither cytosine arabinoside nor aclacinomycin A was furthermore also permitted to as drugs at home from the problem on safety, but the antitumor action of interferon alpha was also expected].
[0015] The evaluation result about a differentiation-inducing operation of a geranyl farnesol can be set to mouse leukemia cell culture cell lineage. Since the evaluation result in Homo sapiens leukemia cell culture cell lineage was not reported at all after that, when the drug sensitivity difference between the cells from which a seed differs was taken into consideration, the effectiveness in Homo sapiens was unknown entirely.
[0016] Thus, the present condition is that there are no drugs which combine the effectiveness which was excellent to various cancers, and safety, and development of the high drugs of usefulness was strongly desired to wide range cancer by clinical.

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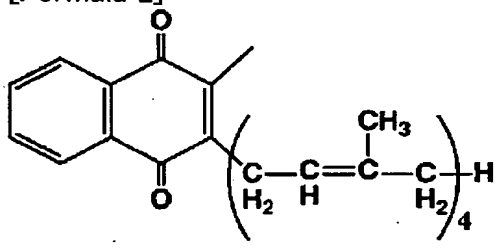
MEANS

[Means for Solving the Problem] The menatetrenone (vitamin K 2) concerning this invention is a vitamin K as a hemostasis vitamin. It is well-known as a compound which has an improvement operation of the following disease by lack, and a symptom, and is widely used by clinical as drugs.
(1) Hypoprothrombinemia by biliary obstruction and the bile hyposecretion (2) Newborn infant hypoprothrombinemia (3) Intrapartum hemorrhage (4) Hypoprothrombinemia which happens during coumarin system anticoagulant administration [0018] There are still few own side effects of menatetrenone, and it is also a compound with very high safety. this invention persons have also examined the effectiveness to a disease besides many years paying attention to menatetrenone having the requirements that the safety to the great bioactive and Homo sapiens, or an animal is high. Consequently, also unexpectedly menatetrenone also had the differentiation-inducing operation, it found out that the desired end could be attained as a therapy / improvement agent to various cancers, such as a hematopoietic organ neoplasm and a solid neoplasm, and this invention was completed. Menatetrenone is expressed with the following chemical structure type.
[0019]
[Formula 2]



[0020] Although had 4 sets of double bonds in intramolecular, a total of eight kinds of geometrical isomers existed two kinds at a time (E bodies, Z body) about 3 sets among those, menatetrenone is set to this invention, the isomer of a gap is sufficient and it is not limited, an all transformer object is more desirable. Moreover, in this invention, one kind in these geometrical isomers may be used independently, two or more kinds of mixture may be used, and it is not limited. The origin is not limited, either, although there are furthermore a natural extract and synthetic compounds in menatetrenone. In addition, menatetrenone is widely sold as drugs, cosmetics, food, an industrial raw material, etc., and can come to hand easily.
[0021] Therefore, the purpose of this invention is to offer therapy / improvement agent to various cancers with the high clinical usefulness which has a differentiation-inducing operation. It is related with therapy / improvement agent of various cancer and malignant tumors, such as a hematopoietic organ neoplasm, a solid neoplasm, etc. which specifically makes menatetrenone an active principle, and therapy / improvement agent of a disease with an effective differentiation-inducing operation of this compound. Here as an example of the concrete disease name of a hematopoietic organ neoplasm Acute leukemia, chronic leukemia, a malignant lymphoma, a multiple myeloma, a macroglobulinemia, etc. can be mentioned. For example, as a solid neoplasm For example, a brain tumor, a head and neck cancer, a breast cancer, lung cancer, an esophagus cancer, gastric cancer, colon cancer, hepatic carcinoma, A gallbladder and a cholangioma, a pancreatic cancer, islet cell cancer, renal cell carcinoma, adrenal cortical adenocarcinoma, vesical cancer, Although a prostatic cancer, the orchioncus, an ovarian cancer, a uterine cancer, a choriocarcinoma, a thyroid cancer, a carcinoid-type-bronchial-adenoma neoplasm, skin carcinoma, a malignant melanoma, an osteosarcoma, a soft tissue sarcoma, a neuroblastoma, a Wilms' tumor, the embryonal rhabdomyosarcomas, a retinoblast kind, etc. can be mentioned It

cannot be overemphasized that the object disease of this invention is not limited to these.
[0022] Moreover, since very high safety is expectable even if it prescribes a medicine for the patient in this invention for a long period of time in addition to the effectiveness as the above-mentioned therapy / improvement agent, it becomes possible to continue a prolonged therapy and it can be said that it is invention which contributes to the improvement of a cancer patient's quality of life greatly.
[0023] Next, in order to show the safety of menatetrenone, an acute toxicity test test result (fifty percent lethal dose value) is shown.
[Table 1]
メナテトレノンの急性毒性 (mg/Kg)

動物種	性別	経口	皮下	腹腔内
マウス (ICR系)	♂	> 5,000	> 5,000	> 5,000
	♀	> 5,000	> 5,000	> 5,000
ラット (SD系)	♂	> 5,000	> 5,000	> 5,000
	♀	> 5,000	> 5,000	> 5,000

[0024] The very high safety of menatetrenone is clear from Table 1. Menatetrenone is already used by clinical widely as drugs still as mentioned above, and the safety is checked.
[0025] As an administration pharmaceutical form, oral pharmaceutical preparation, such as powder, a fine grain agent, a granule, a tablet, a covering tablet, and a capsule, injection pharmaceutical preparation, and external preparations (endermic pharmaceutical preparation) are mentioned, for example. In the case of pharmaceutical-preparation-izing, it can manufacture with a conventional method using the usual pharmaceutical preparation support.
[0026] That is, in order to manufacture oral pharmaceutical preparation, menatetrenone, an excipient, and after adding a binder, disintegrator, lubricant, a coloring agent, correctives, etc. if needed further, it considers as powder, a fine grain agent, a granule, a tablet, a covering tablet, a capsule, etc. with a conventional method.
[0027] As an excipient, a lactose, corn starch, white soft sugar, grape sugar, a mannitol, a sorbitol, crystalline cellulose, a silicon dioxide, etc., for example as a binder For example, polyvinyl alcohol, polyvinyl ether, methyl cellulose, Ethyl cellulose, gum arabic, tragacanth, gelatin, a shellac, The hydroxypropyl methylcellulose, hydroxypropylcellulose, A polyvinyl pyrrolidone, polypropylene-glycol polyoxyethylene block polymer, meglumine, etc. as disintegrator For example, starch, an agar, the end of gelatin, crystalline cellulose, a calcium carbonate, A sodium hydrogencarbonate, calcium citrate, a dextrin, pectin, carboxymethyl-cellulose calcium, etc. as lubricant For example, what is permitted that magnesium stearate, talc, a polyethylene glycol, a silica, hardening vegetable oil, etc. add in drugs as a coloring agent is used for the menthol, aromatic powder, mentha oil, camphor Borneo, a cinnamomi cortex pulveratus, etc. as correctives in the end of cocoa. Of course, these tablets and granules are not hindered by coating suitably according to glycocalyx and other need.
[0028] Moreover, in case the pharmaceutical preparation for injection is manufactured, a solubilizing agent, a stabilizing agent, etc. are added to menatetrenone pH regulator, a resolvent, an isotonizing agent, etc. and if needed, and it pharmaceutical-preparation-izes with a conventional method.
[0029] The approach at the time of manufacturing external preparations is not limited, but can be manufactured with a conventional method. That is, it is possible to use the various raw materials usually used for drugs, quasi drugs, cosmetics, etc. as a base ingredient used in pharmaceutical-preparation-izing.
[0030] As a base ingredient to be used, specifically For example, animal and vegetable oils, straight mineral oil, ester oil, Waxes, higher alcohol, fatty acids, silicone oil, a surfactant, Although raw materials, such as phospholipid, alcohols, polyhydric alcohol, water soluble polymers, clay minerals, and purified water, are mentioned and pH regulator, an anti-oxidant, a chelating agent, a preservation-from-decay antifungal agent, a coloring agent, perfume, etc. can be added further if needed The base ingredient of the external preparations concerning this invention is not limited to these. Moreover, components, such as the component which has other differentiation-inducing operations if needed, a blood-flow accelerator, a germicide, an antiphlogistic, a cell activator, vitamins, amino acid, a moisturizer, and a keratolytic drug, can also be blended. In addition, the addition of the above-mentioned base ingredient is an amount which becomes the concentration usually set up in manufacture of external preparations.
[0031] although the clinical doses of the menatetrenone in this invention differ, and are not limited by a symptom, severity, age, complication, etc. and it changes with the class, routes of administration, etc. of a compound — usually — an adult — 10mg - 10g per day it is — desirable — 50mg - 5g — it is — further —

desirable — 100mg - 1g — it is — this — the inside of taking orally and a vein — or dermal administration is carried out.

[0032] Next, although an example is hung up over below in order to explain this invention concretely, it cannot be overemphasized that this invention is not limited to these.

[0033]

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EXAMPLE

[Example]
Example 1 Granule [0034]
[Table 2]
<処方>

	原料	配合量（mg）
1)	メナテトレノン	100.0
2)	無水ケイ酸	100.0
3)	D-マンニトール	450.0
4)	ヒドロキシプロピルセルロース	40.0
5)	dl-α-トコフェロール	0.2
6)	タルク	10.0
7)	乳糖	約 300.0

[0035] Example 2 Tablet [0036]
[Table 3]
<処方>

	原料	配合量（mg）
1)	メナテトレノン	10.0
2)	ヒドロキシプロピルセルロース	50.0
3)	乳糖	100.0
4)	トウモロコシデンプン	20.0
5)	無水ケイ酸	3.0
6)	ステアリン酸マグネシウム	0.2
7)	マクロゴール6000	3.0
8)	ポリビニルピロリドン	0.6
9)	アラビアゴム末	3.0
10)	沈降炭酸カルシウム	4.0
11)	酸化チタン	10.0
12)	タルク	15.0
13)	白糖	約 60.0

[0037] Example 3 Injections [0038]
[Table 4]
<処方>

	原料	配合量（重量%）
1)	メナテトレノン	1.0
2)	ポリオキシエチレンソルビタンモノオレート	3.5
3)	D-ソルビトール	5.0
4)	リン酸二水素ナトリウム（NaH_2PO_4）	0.08
5)	リン酸水素ナトリウム（Na_2HPO_4）	0.07
6)	精製水	加えて100.0

[0039] Example 4 External preparations [0040]
[Table 5]

<処方>

原料	配合量（重量%）
1) メナテトレノン	1．0
2) スクワラン	10．0
3) ミリスチン酸イソプロピル	7．0
4) ベヘニルアルコール	1．0
5) セトステアリルアルコール	5．5
6) ステアリン酸モノグリセリン	2．0
7) d－α－トコフェロール	0．05
8) ＰＯＥ（２０）モノステアリン酸ソルビタン	2．0
9) キサンタンガム	0．1
10) 1、3－ブチレングリコール	2．0
11) グリセリン	3．0
12) D-ソルビトール	5．0
13) パラベン	0．2
14) 精製水	加えて１００．０

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Front myelogenous leukemia cell HL60 It is drawing having shown the relation of the receiving menatetrenone concentration and the differentiation-inducing operation. (n= 3 and an average ** standard error also show each group)
[Drawing 2] The Homo sapiens monoblast U937 It is drawing having shown the relation of the menatetrenone concentration and the differentiation-inducing operation over a cell. (n= 3 and an average ** standard error also show each group)
[Drawing 3] Homo sapiens myeloblast Mr. leukemic cell It is drawing to ML1 having shown menatetrenone concentration and the relation of a differentiation-inducing operation. (n= 3 and an average ** standard error also show each group)
[Drawing 4] Homo sapiens bone marrow erythroblast leukemic cell K562 It is drawing having shown the relation of the receiving menatetrenone concentration and the differentiation-inducing operation. (n= 3 and an average ** standard error also show each group)

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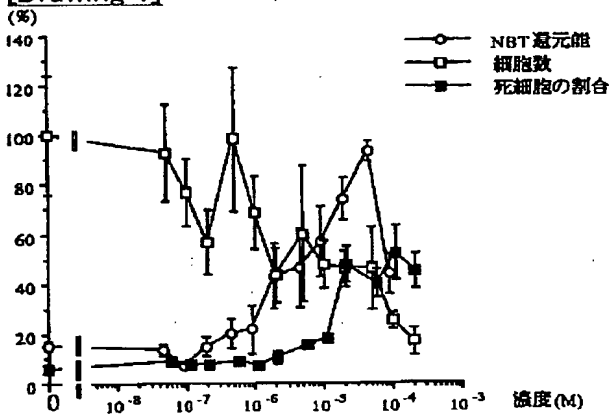
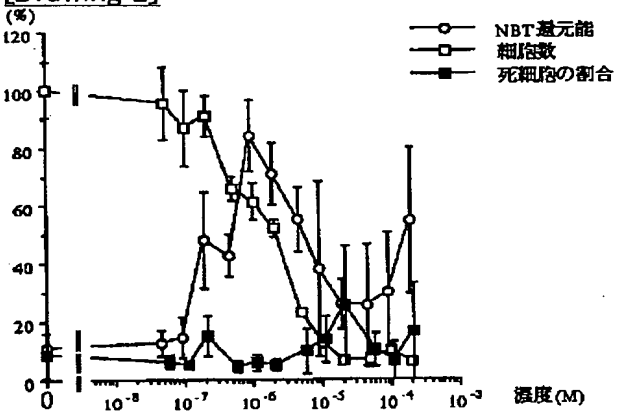
DRAWINGS

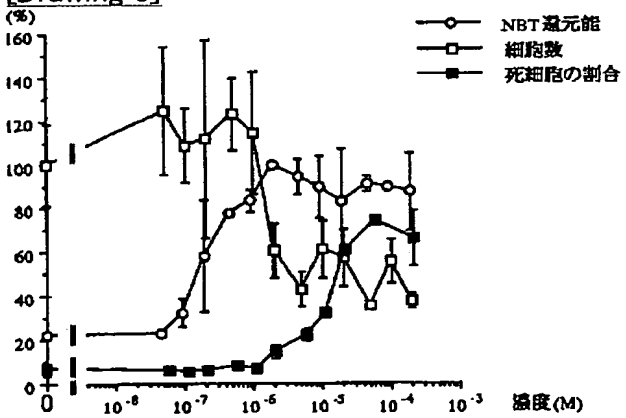
[Drawing 1]



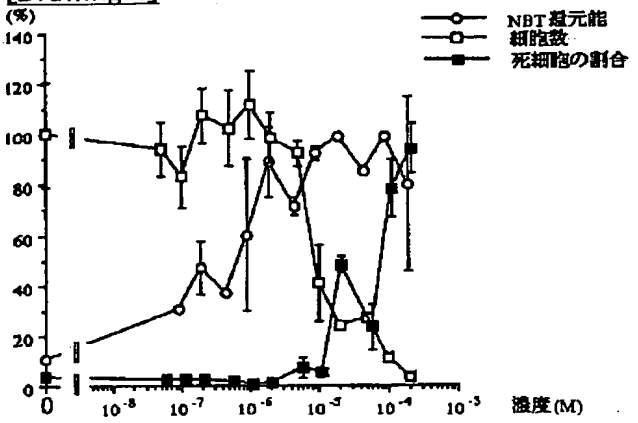
[Drawing 2]



[Drawing 3]



[Drawing 4]



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